LIPOSOMAL COMPOSITIONS AND METHODS OF PREPARATION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application is a continuation of U.S. Serial No. 09/646,994, filed November 20, 2000, which is the National Stage of PCT/US99/06959 filed March 30, 1999, the entire contents of which are hereby incorporated.

FIELD OF THE IVENTION

[0002] The present invention is directed to liposomal compositions of local anesthetics, such as bupivacaine, and in particular to DRV (dehydration-rehydration vesicle) compositions and methods of their preparation and use.

BACKGROUND OF THE INVENTION

[0003] Long acting local anesthetic formulations hold great promise for the management of acute pain, as long lasting analgesia could be achieved with a single dose administered after surgery or trauma. Liposomal local anesthetic formulations have been shown to prolong analgesic duration in animals (Grant et al.; Mowat et al., Boogaerts, Declercq et al., 1993) and humans (Boogaerts, Lafont et al., 1996). The slow release of drug from the liposomal depot decreases the potential for systemic toxicity, and allows for administration of a greater dose. The effectiveness of liposomal bunivacaine in providing prolonged analgesia in a rat postoperative wound

model has been demonstrated (Grant el al.). Formulations described to date, however, have drug-to-lipid ratios, which are too low to be clinically useful in humans.

[0004] Further issues must also be resolved before liposomal local anesthetics can be used to manage acute pain in patients. Critical requirements for a liposomal formulation include reliability and reproducibility in manufacturing and performance, and adequate shelf stability to permit long term storage. Liposomal local anesthetic compositions, which have been described in the literature, employ multilamellar vesicles (MLV) and large unilamellar vesicles (LUV). These liposomes are stored in aqueous media, whereby the component lipids are subject to degradation due to oxidation and hydrolysis. Moreover, encapsulated drug may leak from the liposome into the aqueous medium.

[0005] It is therefore desirable to provide a liposomal anesthetic formulation which has a high loading of drug, relative to total lipids and to total volume, and which has long term storage stability.

SUMMARY OF THE INVENTION

[0006] In one aspect, the invention provides a method of providing long term local anesthesia, comprising administering to a subject in need of such treatment a liposomal local anesthetic formulation prepared by the dehydration-rehydration

method, in which lyophilized liposomes encapsulating the local anesthetic are rehydrated by agitating in an aqueous medium. Preferably, the preparation includes the further step of washing the rehydrated liposomes in hyperosmotic saline solution. An exemplary local anesthetic is bupivacaine. Other local anesthetics which may be used, as discussed further below, include lidocaine, ropivacaine, levobupivacaine, procaine, chloroprocaine, benzocaine, etidocaine, mepivacaine, prilocaine, ciprocaine, tetracaine, dibucaine, heptacaine, mesocaine, propanocaine, carbisocaine, and butacaine.

[0007] More generally, the invention provides a method of preparing a liposomal drug composition having a high drug/lipid ratio, comprising the steps of encapsulating the drug in liposomes, lyophilizing the liposomes, rehydrating the lyophilized liposomes by agitating in an aqueous medium, and washing the rehydrated liposomes in hyperosmotic saline solution. In one embodiment, the drug is a local anesthetic, such as bupivacaine.

[0008] In a related aspect, the invention provides a liposomal local anesthetic composition having a high drug/lipid ratio, where the local anesthetic is preferably bupivacaine, and the drug/lipid ratio is preferably at least 0.3 (mole/mole), and more preferably at least 0.33

(mole/mole). Such liposomal local anesthetic compositions are prepared by encapsulating the local anesthetic in liposornes, lyophilizing the liposornes, and rehydrating the lyophilized liposomes by agitating in an aqueous medium, and washing the rehydrated liposomes in hyperosmotic saline solution.

BREIF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 shows the duration of sensory block observed in mice after administration of 0. 5 % free, 0.5% liposomal (DRV), and 3.5% liposomal bupivacaine (BUP).

[0010] Figure 2 shows the amount of bupivacaine remaining at the site of injection at a series of time intervals after administration of the compositions of Fig. 1.

[0011] Figure 3 is a kinetic release profile of the free and liposomal bupivacaine formulations, showing the data of Figure 2 expressed as a percentage of initial dose.

DETAILED DESCRIPTION OF THE INVENTION

I. DRV (Dehydration-Rehydration) Liposomes

A. Preparation

[0012] Bupivacaine hydrochloride (BUP) (Sigma, St. Louis, MO) was encapsulated into liposomes by the dehydration-rehydration (DRV) technique, as described, for example, in *Kirby et al.* Dehydrated-rehydrated vesicles (DRVs) are liposomes which can be reproducibly prepared, stored in a lyophilized state, and rehydrated immediately prior to

administration (Kirby). Maintaining the formulations in the dehydrated state greatly reduces the likelihood of degradation, and confers shelf stability (Zuidam).

[0013] Two different matrix lipids were used: 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPQ and distearoyl-sn-glycero-3-phosphatidylcholine (DSPQ (Avanti Polar Lipids, Alabaster, Alabama). DMPC, having a gel-to-liquid-crystalline phase transition temperature (Tm) of 23°C, tends to give a more fluid membrane, while DSPC, having a Tm of 52.5°C, gives a more rigid membrane, as discussed further below.

[0014] Twenty or forty mole % cholesterol (CHOL, Sigma, St. Louis, MO) was used in combination with each phospholipid. Two different molar ratios of BUP/lipid (0.64 and 1.28) were employed, as well as two different pH levels.

[0015] For DRV preparation, DMPC or DSPC and CHOL were co-dissolved in tert-butanol (Fisher, Pittsburgh, PA) and lyophilized. The dried lipid was hydrated with water at 60°C to form multilamellar vesicles (MLV). Small unilamellar vesicles (SUVs) were prepared by highpressure (8,000-10,000 psi) homogenization (Minilab 8.30H, APV Rannie, Albertslund, Denmark). SUV size was confirmed to be approximately 100 nm by photon correlation spectroscopy (N4 Plus, Coulter, Miami, FL). BUP was dissolved in the SUVs at pH 4.0 or 5.5. The

solution was then divided, transferred to glass bottles, frozen and lyophilized overnight.

[0016] To prepare DRV formulations, the lyophilized powder was hydrated with saline while vortexing at 60°C to achieve a final lipid concentration of 10%. Prior to characterization and injection, free drug was removed from the final liposomal formulations by 4 successive centrifugal washings with normal saline at 4°C, followed by a final wash with hyperosmotic saline (580 mM) at 4°C. As noted below, use of this final wash of hyperosmotic saline, e.g. having a concentration of about 300 to 600 mM NaCl, was found to give a very high final concentration of drug in the liposomal formulation.

B. Characterization: Bupivacaine Incorporation

[0017] BUP concentration in the liposomes was determined by high performance liquid chromatography (HPLC). Isopropanol (1:1000) was used to dissolve washed liposomes, and aliquots were injected onto an 8-mm x 100-mm column (Radial-Pak 8NVCN, 4mm, Waters, Milford, MA). A mobile phase of acetonitrile: phosphate buffer, 25 mM, pH 4.0 (75:25) was used, and absorption was measured at a wavelength of 210 nm. The retention time of BUP was approximately 4.7 min. Phospholipid concentration was determined using procedures described in Bartlett or Stewart. The BUP to phospholipid ratio (BUP/PQ was then calculated.

[0018] The size of the multilamellar DRV liposomes was determined by photon correlation spectroscopy spectroscopy (N4 Plus, Coulter, Miami, FL). The size distributions of all formulations were similar; the mean diameter of liposomes of formulation 3 was 1931 ±722 nm.

[0019] All in vitro liposomal characterization procedures were performed in triplicate. Results are summarized in Table 1.

Table 1

Formulation	Matrix Lipid	Mole % Chol	Added Bup, mg (molar ration)	Final Bup concn, mg/ml	Drug to PC ratio, mM/mM
1	DSPC	40	40 (1.28)	17	.116
2	DSPC	40	20 (0.64)	19	.127
3	DSPC	20	40	35	.356
4	DSPC	20	20	29	.257
5	DMPC	40	40	4	.024
6	DMPC	40	20	5	.023

[0020] As shown in the Table, liposomes prepared with DSPC as the matrix lipid (formulations 1-4) had higher BUP/PL ratios. The two BUP concentrations resulted in similar BUP/PL ratios. In preliminary studies, the pH of the solution did not affect final BUP/PL ratio, so all subsequent work was done at pH 5.5.

[0021] The choice of matrix lipid markedly affected BUP/PL ratio, with DSPC resulting in more effective BUP encapsulation than DMPC. As noted above, the higher Tm of DSPC tends to give a more rigid liposome at ambient temperature, which may prevent loss of BUP during washings.

[0022] For DSPC liposomes, lower % cholesterol (formulations 2-3) resulted in greater BUP/PL ratios. Accordingly, still lower levels of cholesterol may be used. For DMPC, on the other hand, lower % cholesterol resulted in very low BUP/PL ratios (data not shown). Cholesterol is known to increase membrane rigidity, and therefore may have reduced leakage of the less rigid DMPC liposomes. In the case of the more rigid DSPC liposomes, it is possible that BUP and CHOL compete for the same sites in the bilayer.

[0023] Formulation 3 gave a drug/PC ratio of about 0.36, which is much higher than previously reported for MLV loaded by standard methods (e.g. about 0. 1, reported by *F. Legros et al.*), and even higher than reported for remote loading using sodium citrate, (about 0.26). A high drug-to-lipid ratio is especially important in treatment of superficial wounds, where a high amount of lipid residue is undesirable.

[0024] Using hyperosmotic saline for the final wash, in place of normal saline, consistently increased the final BUP concentration of the liposomes. Formulation 3 yielded a liposomal BUP concentration of 3.5 % by weight, higher than any previously reported liposomal BUP formulation. The explanation for this finding is not certain, but may be related to a shrinking of liposomes, allowing a greater number

of liposomes into the pellet. The net effect would be an increase in the final BUP concentration.

[0025] It was further observed that the drug in these DRV liposomes appeared to be present in two pools, one membrane-associated and the other in the intraliposomal aqueous phase. The distribution varied with the relative concentration of the lipid and drug used, and ratios in the range of 10: 1 to 40:60 (membrane to intraliposomal aqueous phase) were observed. Because the two pools release the drug by different mechanisms, each at a different rate, this distribution could be manipulated to control the rate of release of the drug.

II. Analgesic Efficacy

[0026] The formulation which yielded the greatest BUP/PL ratio (formulation 3) was evaluated in vivo to determine analgesic efficacy. All experiments were approved by the Institutional Animal Care and Use Committee. Male Swiss-Webster mice weighing 26 + 3 g (mean + SD) were used. Animals had free access to food and water, and were maintained on 12-hour dark-light cycle. Prior to testing, the hair overlying the abdomen was shaved. Analgesia was assessed using response to cutaneous electrical stimulation. A current generator (model S48, Grass Instruments, Quincy, MA) coupled to a constant current unit (model PSIU6F, Grass Instruments, Quincy, MA) was used. The current was delivered to the skin

surface by touching it gently with two electrodes fashioned from #25g needles. The vocalization threshold (the current required to produce a vocalization response) was assessed prior to injection of study solutions. This was done by administering two successive stimuli (1 Hz), beginning at 1-mA and increasing in 1-mA increments to a cut-off of 15 mA. Mice who failed to vocalize at 15 mA were excluded from the study.

[0027] To determine analgesic duration, mice were injected with liposomal BUP compositions having 0.5 wt % and 3.5 wt % bupivacaine, respectively, formed by dilution (as necessary) of formulation 3, above. Free BUP (0.5 %), hyperosmotic saline (580 mM), or drug-free liposomes were used as controls. For all groups, 150ml of test solution was injected subcutaneously using a #25g needle in 8 mice. After injection, sensory block was assessed at 5, 15, 30 minutes and then at 1, 1.5, 2, 3, 4.5, 6, 9, 12, 14, 16, 17, and 19 hours. Failure to vocalize in response to stimulation with threshold current was taken as analgesia. Testing was continued until two successive tests resulted in vocalization (i.e., absence of analgesia). Duration of sensory block was determined using Kruskall Wallis, with $p < 0.05$ considered significant. Results are presented in Figure 1.

[0028] As shown in the Figure, in mice receiving 0.5% free BUP, analgesia was exhibited at 30 minutes, but none was

detected at 2 hours. In mice given 0.5 % liposomal BUP, all animals demonstrated analgesia at 3 hours, and it was not until 6 hours that analgesia was not detected. For the 3.5 % liposomal BUP formulation, all animals had sensory block for 14 hours, and it did not regress in all animals until 19 hours. Thus, a 6 fold increase in analgesic duration was observed when a 0.5% concentration of BUP was injected in liposomes, and this increased to a 28 fold increase when 3.5% liposomal BUP was injected.

[0029] Significantly, no obvious signs of toxicity were apparent at this dose, whereas systemic toxic effects of free BUP precluded the administration of doses greater than 0.5%, as they produced toxicity in preliminary studies.

III. Tissue BUP Concentration Post-Administration

[0030] In a separate group of mice, the amount of drug remaining at the site of injection after administration of 0.5% and 3.5% liposomal BUP (formulation 3) or 0.5% free BUP was determined. Three animals were sacrificed at 0, 0.25, 0.5, 1, 2, 4, and 8 hours after injections of all drug formulations and also at 16, 24, 48 and 96 hours after injection of the liposomal formulations. After sacrifice, a 1-cm2 circular tissue section including the entire area of injection, and extending to the peritoneum, was excised. The tissue was homogenized in 1 ml of isopropanol for 1 min, and

then centrifuged at 16,000 x G (Eppendorf Centrifuge 5417C, Engelsdorf, Germany). The supernatant was diluted 1:100 in isopropanol, and BUP concentration was determined using HPLC. *In vivo* release kinetics were done in triplicate. Results are presented in Fig. 2, where the absolute amounts remaining are expressed as mean ± SD. Figure 3 shows the data expressed as percent of initial dose.

[0031] As shown in the Figures, free BUP was rapidly redistributed from the injection site, as compared to the liposomal BUP formulations. Four hours after injection, only 1% of the injected dose of free BUP remained at the site (8/750 mg), whereas approximately 54% and 66% of the 0.5% and 3.5% liposomal formulations remained (406/750 mg and 3483/5250 mg, respectively). The redistribution profiles of the 0.5% and 3.5% liposomal BUP formulations were similar. The $t_{1/2}$ was 6.5 hours for the 0.5% liposomal BUP formulation and 6.8 hours for 3.5% liposomal BUP. These similar release profiles, shown in Fig. 3 as percent of injected dose, extended to 96 hours, where 16.8% (126 mg) and 16.5% (868 mg) of the 0.5% and 3.5% liposomal BUP formulations were recovered, respectively.

[0032] The slow release of drug from the liposomes provides the benefits of 1) a prolonged residence of drug at the site of injection and 2) a decrease in systemic drug availability. A comparison of Figures 2 and 3 shows that much of the

injected drug remained associated with the liposomal vehicles after analgesia was no longer exhibited. It is possible that the continued release of BUP produced a weak analgesic effect, which was not detected by our testing technique. Future DRV studies could be directed at decreasing the size of this drug reservoir and achieving more consistent release, perhaps by the use of smaller liposomes. The DRV liposomes in the current study were large, with a mean diameter approximately 2 mm. Although larger liposomes tend to remain longer at the site of injection; a smaller liposome size could result in more thorough release of BUP and less retention in the depot after the analgesic effect has subsided.

[0033] The liposomal BUP formulations described herein significantly prolonged the duration of sensory block after subcutaneous administration, in comparison to free bupivacaine. The present liposomal formulations are distinguished in that they are formed by the DRV technique, thus giving long storage stability, preferably employ DSPC or another high Tm lipid, and have a high (i.e. greater than about 0.15, preferably greater than about 0.25, and more preferably greater than about 0.35) drug-to-lipid ratio. Preparation of the formulations employs a hyperosmotic saline wash, as described above, which results in a high concentration of drug in the final emulsion.

[0034] Other local anesthetics may also be used in these formulations. A "local anesthetic", as used herein, refers to a member of the "caine" family of local anesthetic compounds, having physicochemical properties similar to bupivacaine. Structural features common to these compounds include a benzene ring linked via an ester or amide linkage, or, less frequently, a carbamate linkage, to an aliphatic or alicyclic group containing a tertiary or quaternary amine. The length of the linkage, i.e. the distance between the benzene ring and the amine, is typically about 6-9 Å. These compounds include, for example, lidocaine, ropivacaine, levobupivacaine, procaine, chloroprocaine, benzocaine, etidocaine, mepivacaine, prilocaine, ciprocaine, tetracaine, dibucaine, heptacaine, mesocaine, propanocaine, carbisocaine, and butacaine.

[0035] The compositions may be administered subcutaneously, intraperitoneally, topically, and into the neuraxis. Methods for preparing suitable dosage forms are known or will be apparent to those skilled in the art; for example, see *Remington's Pharmaceutical Sciences* (19th Ed., Williams & Wilkins, 1995). The composition to be administered will contain a quantity of bupivacaine in a pharmaceutically effective amount for effecting anesthesia in the subject. Such formulations may allow a single injection to provide prolonged analgesia, and thus obviate the need for repeated

injections, catheterization, or use of an infusion pump, as are commonly required with conventional anesthetics. Administration could be performed during or at the end of surgery, and by providing effective pain release could obviate the need for parental narcotics.

[0036] While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications may be made without departing from the invention.

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